Development of a cost-effective basal medium for *in-vitro* propagation of Anthurium (*Anthurium andreanum*) as an alternative for Murashige and Skoog (MS) medium

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Abstract

Generally, Murashige and Skoog (MS) medium is used for in-vitro micropropagation of Anthurium but it is very costly. Therefore the major objective of this study was to develop a cost-effective basal medium for micropropagation of Anthurium as an alternative to MS medium. In the present experiment, Albert's solution, which is a popular fertilizer mixture, developed for hydroponics systems was used. All experiments were arranged according to a completely randomized design with ten replicates. 2 kg of Albert's mixture in 25 L water was used as stock. Albert's solution (1 ml of stock in 1L of water) with four different pH values (i.e. 5.0, 5.2, 5.4 and 5.6) was compared with MS medium for culture establishment. Five different levels of Albert's solution (1, 2, 3, 4 and 5 ml/L) were tested for in-vitro embryo establishment. Germination percentage was recorded 10 days after inoculation. Then 10 different concentrations of Albert's solution (i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml/L) were compared for in-vitro multiplication and rooting. The hormones 1.5 mg/ L BAP and 0.5 mg/ L 2, 4-D were supplemented for multiplication while 1 mg/ L BAP and 0.5 mg/L IBA were added for rooting. Three months after inoculation, number of shoots, roots and leaves per shoot were recorded.

The highest germination percentage (90%) was observed on Albert's medium with pH 5.4. The highest germination rate (85%) was recorded in 3 ml/L concentration of Albert's medium. After three months the highest shoot number (48.4) was recorded in MS media, which was not significantly different from 5 ml/L concentration of Albert's medium at $p \leq 0.05$. The significantly highest number of roots per plant (15.1) was observed in 7 ml/L of concentration level while it was about 8 in the control. The highest number of leaves per shoot (9.4) was observed on Albert's medium at a concentration of 5 ml/L. Leaves became yellowish color in 8, 9 and 10 ml/ L concentration of Albert's medium. Survival rate of acclimatized plantlets obtained from Albert's medium was about 90% compared to MS medium (65%).

The total cost for 1 L MS media was about Rs. 225/- and the total cost of Albert's medium was about Rs. 110/-. It could be concluded that Albert's solution is a cost-effective alternative medium for MS for in-vitro propagation of Anthurium.

Key words: *Anthurium andraeanum*, Murashige and Skoog medium, Albert's solution

Introduction

Tissue culture technique needs special skills and knowledge, especially for preparation of artificial medium, explant selection, sterilization, culture establishment and multiplication etc. Considering medium preparation, all macronutrients, micronutrients, vitamins, hormones and sugar compounds should be included in correct amounts; otherwise plants will not grow well. Albert's solution contains 11% N, 8% P_2O_5, 15% K_2O, 14% MgO, Fe, Mn, B, Zn, Cu and Mo in balanced amounts. However, it does not contain sugar, vitamins and hormones. Therefore a series of experiments were conducted to develop a cost effective basal medium using Albert's solution for *in-vitro* propagation of Anthurium.

Methodology

The research study was conducted at the tissue culture laboratory, Department of Crop Science, Faculty of Agriculture, University of Ruhuna. All experiments were set up according to a completely randomized design with ten replicates. A stock of Albert's solution was prepared (2 kg of Albert's mixture dissolved in 25 L of water). 8 g/L Agar was added to the medium for solidification and Murashige and Skoog (MS) medium (Murashige, 1962) was used as the control.

Step 1: Selection of proper pH value for culture establishment

1 ml of stock solution was dissolved in 1 L distilled water and pH was adjusted to 5.0, 5.2, 5.4 and 5.6 using 0.1 NaOH and 0.1 HCl. Surface sterilized seeds were inoculated in each treatment and after 10 days, survival percentages of seeds were observed. Phytohormones were not used and MS medium was used as the control.

Step 2: Selection of suitable concentration of Albert's solution for embryo establishment

Mature seeds of Anthurium were used to isolate embryos. 5 concentration levels of Albert's solution (1 ml, 2 ml, 3 ml, 4 ml and 5 ml stock solution were mixed with 1 L distilled water) were used and 3% sucrose was added to each concentration. 2 mg/L 2-4, D and 0.8 mg/L NAA were added to all treatments. MS medium supplemented with the same hormone and 3% sucrose was used as a control. Surface sterilized seeds were inoculated in each treatment. pH value was adjusted to 5.4 by using HCl and NaOH. After 10 days, germination percentages were recorded.

Step 3: Best concentration of Albert's solution for multiplication and rooting

Ten levels of concentrations were compared. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml of stock solution were dissolved in 1 L distilled water separately. Recommended rates of MS vitamins (i.e. nicotinic acid, thiamine, pyridoxine and glycine) and 1.5 mg/L of BAP and 0.5 mg/ L 2, 4-D were added for *in vitro* multiplication. For rooting 0.1 mg/L BAP and 0.5 mg/L IBA were supplemented to the medium. MS medium supplemented with the same hormone levels were used as a control treatment. pH value was adjusted to 5.4 by using HCl and NaOH. *In-vitro* grown shoots were used as explants. Sub-culturing was done, using the same medium at one month intervals. Number of roots, number of shoots and number of leaves on shoot were recorded during sub-culturing.

Step 4: Potting mixture for acclimatization

Sterilized sand and coir dust were used with different ratios as follows:

	Sand		**Coir dust**
T_1	1	:	1
T_2	2	:	1
T_3	1	:	2
T_4	3	:	1
T_5	1	:	3

Rooted plantlets grown in MS and Albert's medium were acclimatized. During acclimatization, 100% humidity was maintained in the chamber up to three weeks and humidity was decreased gradually after three weeks.

Results and discussion

Highest germination percentage (90%) was observed in Albert's medium with pH 5.4. Only 25% and 35% germination percentages were recorded in media with pH 5.0 and 5.6 respectively. It may be due to nutrient unavailability for absorption of plants with decreasing or increasing pH value (Figure 1).

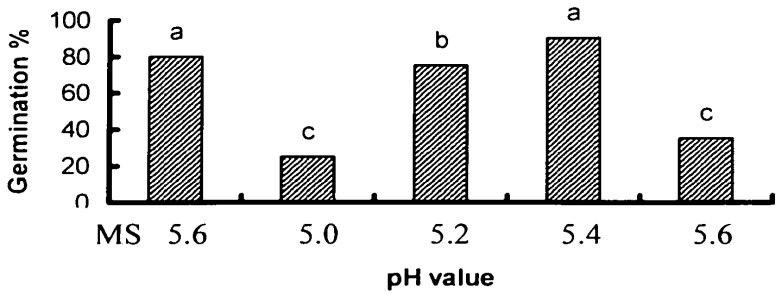


Figure 1: Germination percentage with different pH values of Albert's solution and MS medium Means on the columns with same letter are not significantly different at ($p \leq 0.05$)

The medium with a concentration of 3 ml/L showed the highest germination rate (85%) and the highest survival percentage (80%) while in the control, germination rate and survival percentage were 70% and 75% respectively (Figure 2).

Highest mortality rate (85%) was recorded in 5 ml/L concentration of Albert's solution. It may be due to toxic effects of the nutrients on embryo growth. On the other hand very poor growth performances were recorded in 1 ml/L treatment due to lack of essential nutrients with deceasing concentrations.

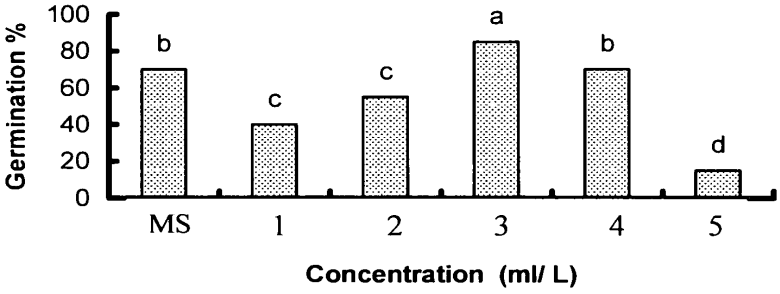
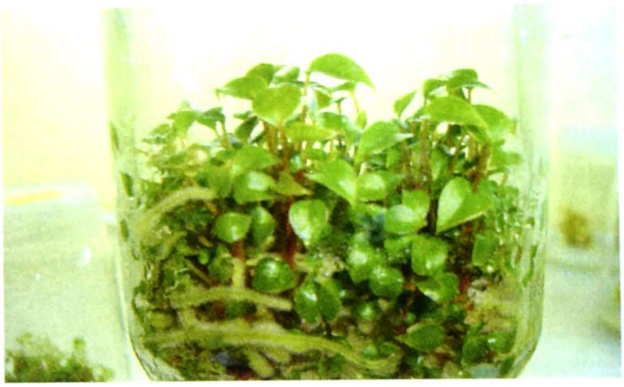


Figure 2: Germination percentage with different concentrations of Albert's solution. Means on the columns with same letter are not significantly different at ($p \leq 0.05$)

After three months, highest mean shoot number (48.4) was recorded on MS medium (Plate 1). It was not significantly different from 5 ml/L concentration level (47.5) Albert's solution. The highest mean number of roots per plant (15.1) was observed in 7 ml/L of Albert's solution; while on MS it was only about 8. The highest mean number of leaves per shoot (9.4) was observed in medium with 5 ml/ L concentration of Albert's solution (Table 1). Approximately 5 leaves per shoot were recorded in the control treatment. Leaves turned yellowish color when concentration of Albert's solution increased from 8 ml/L to 10 ml/L.

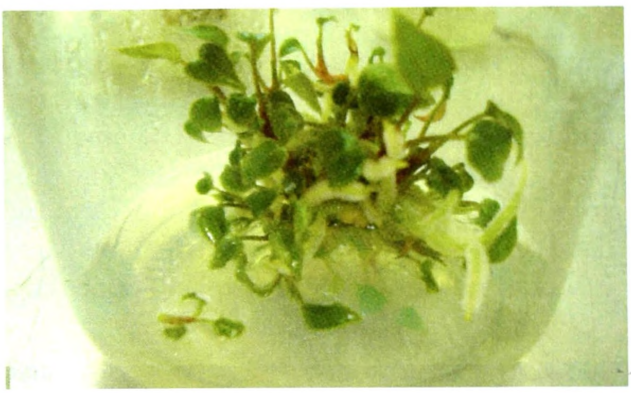
Two weeks after acclimatization, the highest survival rate was observed in Sand: Coir dust 1:2 medium. Mortality rate was increased with increasing coir dust in the medium. It may be due to high water content in the medium.

A



B

C



D

E

Plate 1

A Multiplied shoots in MS medium
B Best concentration of Albert's medium (5 ml/ L) for shoot multiplication
C Best concentration of Albert's medium (7 ml/ L) for rooting
D Rooting was shown in MS medium
E Acclimatized plantlets after three weeks in Sand: Coir dust: 1:2 medium

Table 1. Mean number of shoots, roots and leaves per shoot Means on the columns with same letter are not significantly different at ($p \leq 0.05$)

Treatments	Mean number of		
	Shoots	Roots	Leaves per shoot
Control (MS)	48.4^{a}	8.6^{c}	5.6^{c}
1 ml/L (T_1)	11.2^{f}	2.5^{g}	2.0^{fg}
2 ml/L (T_2)	14.8^{ef}	2.7^{g}	3.0^{e}
3 ml/L (T_3)	27.0^{d}	3.0^{f}	4.7^{d}
4 ml/L (T_4)	30.8^{c}	3.6^{f}	6.8^{b}
5 ml/L (T_5)	47.5^{a}	5.3^{de}	8.6^{a}
6 ml/L (T_6)	38.7^{b}	6.8^{d}	4.4^{d}
7 ml/L (T_7)	35.8^{b}	15.1^{a}	2.9^{f}
8 ml/L (T_8)	22.4^{de}	10.2^{b}	3.8^{e}
9 ml/L (T_9)	11.6^{fg}	5.7^{de}	1.8^{g}
10 ml/L (T_{10})	5.7^{h}	6.4^{d}	2.6^{f}

Conclusions

The best pH value was 5.4 and the best concentration of Albert's solution was 3 ml/L with 2 mg/L of 2, 4 -D and 0.8 mg/L of NAA for embryo establishment while best concentration of Albert's solution was 5 ml/L with 1.5 mg/L of BAP and 0.5 mg/L 2, 4- D for multiplication. Maximum rooting was seen on 7 ml/L Albert's solution with 0.1 mg/L of BAP and 0.5 mg/L of IBA. Survival rate of acclimatized plantlets obtained from Albert's medium was about 90% compared to MS medium (65%).

The total cost for 1 L MS medium was about Rs. 225/- (Yapabandara *et a.,l* 2005) and the total cost of 1 L Albert's media was only about Rs. 110/-. It could be concluded that Albert's solution is a cost-effective alternative medium for MS for *in-vitro* propagation of Anthurium.

References

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